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File 5:Biosis Previews(R) 1926-2011/Mar W4
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Set	Items	Description
? s ricin and glycosyl?		
	3738	RICIN
	70543	GLYCOSYL?
S1	145	RICIN AND GLYCOSYL?
? s s1 and mutat?		
	145	S1
	468759	MUTAT?
S2	17	S1 AND MUTAT?

? t s2/7/1-17

2/7/1
DIALOG(R)File 5:Biosis Previews(R)

0021164751 BIOSIS NO.: 200900506188
A Human Embryonic Kidney 293T Cell Line %%%Mutated%%% at the Golgi alpha-Mannosidase II Locus
AUTHOR: Crispin Max; Chang Veronica T; Harvey David J; Dwek Raymond A; Evans Edward J; Stuart David I; Jones E Yvonne; Lord J Michael; Spooner Robert A (Reprint); Davis Simon J
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JOURNAL: Journal of Biological Chemistry 284 (32): p21684-21695 AUG 7 2009 2009
ITEM IDENTIFIER: doi:10.1074/jbc.M109.006254
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Disruption of Golgi alpha-mannosidase II activity can result in type II congenital dyserythropoietic anemia and induce lupus-like autoimmunity in mice. Here, we isolated a mutant human embryonic kidney (HEK) 293T cell line called Lec36, which displays sensitivity to %%%ricin%%% that lies between the parental HEK 293T cells, in which the secreted and membrane-expressed proteins are dominated by complex-type %%%glycosylation%%%, and 293S Lec1 cells, which produce only oligomannose-type N-linked glycans. Stem cell marker 19A was transiently expressed in the HEK 293T Lec36 cells and in parental HEK 293T cells with and without the potent Golgi alpha-mannosidase II inhibitor, swainsonine. Negative ion nano-electrospray ionization mass spectra of the 19A N-linked glycans from HEK 293T Lec36 and swainsonine-treated HEK 293T cells were qualitatively indistinguishable and, as shown by collision-induced dissociation spectra, were dominated by hybrid-type %%%glycosylation%%%. Nucleotide sequencing revealed %%%mutations%%% in each allele of MAN2A1, the gene encoding Golgi alpha-mannosidase II: a point %%%mutation%%% that mapped to the active site was found in one allele, and an in-frame deletion of 12 nucleotides was found in the other allele. Expression of the wild type but not the mutant MAN2A1 alleles in

Lec36 cells restored processing of the 19A reporter glycoprotein to complex-type %%%glycosylation%%%. The Lec36 cell line will be useful for expressing therapeutic glycoproteins with hybrid-type glycans and as a sensitive host for detecting %%%mutations%%% in human MAN2A1 causing type II congenital dyserythropoietic anemia.

2/7/2
DIALOG(R)File 5:Biosis Previews(R)

0019935607 BIOSIS NO.: 200700595348
The isolation and characterization of temperature-dependent %%%ricin%%% A chain molecules in Saccharomyces cerevisiae
AUTHOR: Allen Stuart C H; Moore Katherine A H; Marsden Catherine J; Fueloep Vilmos; Moffat Kevin G; Lord J Michael; Ladds Graham; Roberts Lynne M (Reprint)
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JOURNAL: FEBS Journal 274 (21): p5586-5599 NOV 2007 2007
ITEM IDENTIFIER: doi:10.1111/j.1742-4658.2007.06080.x
ISSN: 1742-464X_(print) 1742-4658_(electronic)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Ricin%%% is a heterodimeric plant protein that is potently toxic to mammalian cells. Toxicity results from the catalytic depurination of eukaryotic ribosomes by %%%ricin%%% toxin A chain (RTA) that follows toxin endocytosis to, and translocation across, the endoplasmic reticulum membrane. To ultimately identify proteins required for these later steps in the entry process, it will be useful to express the catalytic subunit within the endoplasmic reticulum of yeast cells in a manner that initially permits cell growth. A subsequent switch in conditions to provoke innate toxin action would permit only those strains containing defects in genes normally essential for toxin retro-translocation, refolding or degradation to survive. As a route to such a screen, several RTA mutants with reduced catalytic activity have previously been isolated. Here we report the use of Saccharomyces cerevisiae to isolate temperature-dependent mutants of endoplasmic reticulum-targeted RTA. Two such toxin mutants with opposing phenotypes were isolated. One mutant RTA (RTAF108L/L151P) allowed the yeast cells that express it to grow at 37 degrees C, whereas the same cells did not grow at 23 degrees C. Both %%%mutations%%% were required for temperature-dependent growth. The second toxin mutant (RTAE177D) allowed cells to grow at 23 degrees C but not at 37 degrees C. Interestingly, RTAE177D has been previously reported to have reduced catalytic activity, but this is the first demonstration of a temperature-sensitive phenotype. To provide a more detailed characterization of these mutants we have investigated their N-%%%glycosylation%%%, stability, catalytic activity and, where appropriate, a three-dimensional structure. The potential utility of these mutants is discussed.

2/7/3
DIALOG(R)File 5:Biosis Previews(R)

17950089 BIOSIS NO.: 200400320846
A novel lectin, DltA, is required for expression of a full serum resistance phenotype in Haemophilus ducreyi
AUTHOR: Leduc Isabelle; Richards Patricia; Davis Crystal; Schilling Birgit; Elkins Christopher (Reprint)
AUTHOR ADDRESS: Sch MedDept Med, Univ N Carolina, 8341C Med Biomol Res Bldg,CB 7031, Chapel Hill, NC, 27599, USA**USA
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JOURNAL: Infection and Immunity 72 (6): p3418-3428 June 2004 2004
MEDIUM: print
ISSN: 0019-9567 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Haemophilus ducreyi, the causative agent of chancroid, is highly resistant to the complement-mediated bactericidal activity of normal human serum (NHS). Previously, we identified DsrA (for ducreyi serum resistance A), a major factor required for expression of the serum resistance phenotype in H. ducreyi. We describe here a second outer membrane protein, DltA (for ducreyi lectin A), which also contributes to serum resistance in H. ducreyi. Isogenic dltA mutants, constructed in 35000HP wild-type and FX517 dsrA backgrounds, were more susceptible to the bactericidal effects of NHS than each respective parent, demonstrating the additive effect of the %%%mutations%%%. Furthermore, expression of dltA in H. influenzae strain Rd rendered this highly susceptible strain partially resistant to 5% NHS compared to a vector-control strain. Although primary basic local alignment search tool analysis of the dltA open reading frame revealed no close bacterial homologue, similarity to the beta-chain of the eukaryotic lectin %%%ricin%%% was noted. DltA shares highly conserved structural motifs with the %%%ricin%%% beta chain, such as cysteines and lectin-binding domains. To determine whether dltA was a lectin, ligand blots and affinity chromatography experiments were performed. DRA was affinity purified on immobilized lactose and N-acetylgalactosamine, and N-%%%glycosylated%%% but not glycosidase-treated model glycoproteins bound DltA. These data indicate that DltA is a lectin with specificity for lactose-related carbohydrates (CHO) and is important for H. ducreyi serum resistance.

2/7/4
DIALOG(R)File 5:Biosis Previews(R)

17664594 BIOSIS NO.: 200400035351
A %%%mutation%%% causing a reduced level of expression of six beta4-galactosyltransferase genes is the basis of the Lec19 CHO %%%glycosylation%%% mutant.
AUTHOR: Lee Jaehoon; Park Sung-Hae; Sundaram Subha; Raju T Shantha; Shaper Nancy L; Stanley Pamela (Reprint)
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JOURNAL: Biochemistry 42 (42): p12349-12357 October 28, 2003 2003

MEDIUM: print
ISSN: 0006-2960 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: To identify factors required for the synthesis of complex glycans, we have isolated Chinese hamster ovary (CHO) cell mutants resistant to plant lectins. We previously identified Lec19 CHO cells as resistant to the Gal-binding lectins %%%ricin%%%, abrin, and modeccin and hypersensitive to the toxicity of other lectins that bind Gal, including L-PHA and E-PHA. Here we show that Lec19 cell extracts have a decreased ability to transfer Gal to simple sugar, oligosaccharide, and glycopeptide acceptors, particularly to biantennary, GlcNAc-terminated acceptors. RicinII-agarose lectin affinity chromatography, oligomapping, and monosaccharide analyses provided evidence that Lec 19 N-glycans have fewer Gal residues than CHO N-glycans. MALDI-TOF mass spectra of N-glycans released from Lec19 cell glycoproteins by peptide N-glycanase F revealed species with the predicted masses of neutral N-glycans with few Gal residues. Such truncated species are essentially absent from CHO cell glycoproteins. However, the complement of fully galactosylated or sialylated bi-, tri-, and tetra-antennary N-glycans was largely equivalent in Lec19 and CHO cells. In addition, the coding region sequences of the beta4GalT-1, -T-2, -T-3, -T-4, -T-5, and -T-6 genes were identical in CHO and Lec19 cells. However, Northern analyses revealed an apprx2-4-fold reduction in the level of transcripts of all six beta4GalT genes in Lec19 cells. Since the recessive Lec19 phenotype is the result of a loss-of-function %%%mutation%%%, the combined data predict the existence of a trans-acting regulator of the steady-state level of transcripts that derive from these six mammalian beta4GalT genes.

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DIALOG(R)File 5:Biosis Previews(R)

17535795 BIOSIS NO.: 200300493452
Induction of direct endosome to endoplasmic reticulum transport in Chinese hamster ovary (CHO) cells (LdlF) with a temperature-sensitive defect in epsilon-coatomer protein (epsilon-COP).
AUTHOR: Llorente Alicia; Lauvrak Silje U; van Deurs Bo; Sandvig Kirsten (Reprint)
AUTHOR ADDRESS: Institute for Cancer Research, Norwegian Radium Hospital, Montebello, 0310, Oslo, Norway**Norway
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JOURNAL: Journal of Biological Chemistry 278 (37): p35850-35855 September 12, 2003 2003
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In the present study we demonstrate that %%%ricin%%%, apparently without passing through the Golgi apparatus, reaches the endoplasmic reticulum (ER) and intoxicates cells in which the Golgi apparatus has been vesiculated by depletion of epsilon-COP, a subunit of COPI. LdlF

cells contain a temperature-sensitive %%%mutation%%% in epsilon-COP. At the nonpermissive temperature epsilon-COP is degraded, and the Golgi apparatus undergoes a morphological change. To study %%%ricin%%% transport in these cells we used %%%ricin%%% sulf-2, a modified %%%ricin%%% molecule containing %%%glycosylation%%% and sulfation sites. Measurements of the incorporation of radioactive mannose into %%%ricin%%% sulf-2 showed that %%%ricin%%% reached the ER in cells depleted of epsilon-COP. Importantly, by investigating the %%%glycosylation%%% of %%%ricin%%% sulf-2 that was modified with radioactive sulfate in the trans-Golgi network, it was demonstrated that transport of %%%ricin%%% to the ER via the Golgi apparatus was severely inhibited. Moreover, we found that %%%ricin%%% was able to intoxicate ldlF cells depleted of epsilon-COP in the presence of brefeldin A. In contrast, control cells were completely protected against %%%ricin%%% by brefeldin A. In conclusion, our results suggest that in ldlF cells depleted of epsilon-COP %%%ricin%%% might be transported to the ER by an induced brefeldin A-resistant pathway that circumvents the Golgi apparatus.

2/7/6

16415999 BIOSIS NO.: 200200009510
Structural and functional studies of cinnamomin, a new type II ribosome-inactivating protein isolated from the seeds of the camphor tree
AUTHOR: Xie Liang; Wang Bao-Zhong; Hu Rong-gui; Ji Hong-bin; Zhang Li; Liu Wang-Yi (Reprint)
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JOURNAL: European Journal of Biochemistry 268 (22): p5723-5733 November, 2001 2001
MEDIUM: print
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cinnamomin is a new type II ribosome-inactivating protein (RIP). Its A-chain exhibits RNA N-glycosidase activity to inactivate the ribosome and thus inhibit protein synthesis, whereas the %%%glycosylated%%% B-chain is a lectin. The primary structure of cinnamomin, which exhibits approximately 55% identity with those of %%%ricin%%% and abrin, was deduced from the nucleotide sequences of cDNAs of cinnamomin A- and B-chains. It is composed of a total of 549 amino-acid residues: 271 residues in the A-chain, a 14-residue linker and 264 residues in the B-chain. To explore its biological function, the cinnamomin A-chain was expressed in Escherichia coli with a yield of 100 mg per L of culture, and purified through two-step column chromatography. After renaturation, the recovery of the enzyme activity of the expressed A-chain was 80% of that of native A-chain. Based on the modeling of the three-dimensional structure of the A-chain, the functional roles of five amino acids and the only cysteine residues were investigated by site-directed mutagenesis or chemical modification. The conserved single %%%mutation%%% of the five amino-acid residues led to 8-50-fold losses of enzymatic activity, suggesting that these residues were crucial for

maintaining the RNA N-glycosidase activity of the A-chain. Most interestingly, the strong electric charge introduced at the position of the single cysteine in A-chain seemed to play a role in enzyme/substrate binding.

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DIALOG(R)File 5:Biosis Previews(R)

14935113 BIOSIS NO.: 199900194773
Structure-function analysis of the UDP-N-acetyl-D-galactosamine: Polypeptide N-acetylgalactosaminyltransferase: Essential residues lie in a predicted active site cleft resembling a lactose repressor fold
AUTHOR: Hagen Fred K; Hazes Bart; Raffo Roberto; Desa Deborah; Tabak Lawrence A (Reprint)
AUTHOR ADDRESS: Center Oral Biology, Rochester Institute Biomedical Sciences, University Rochester, Rochester, NY 14642, USA**USA
JOURNAL: Journal of Biological Chemistry 274 (10): p6797-6803 March 5, 1999 1999
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Mucin-type O-%%%glycosylation%%% is initiated by a family of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases (ppGaNTases). Based on sequence relationships with divergent proteins, the ppGaNTases can be subdivided into three putative domains: each putative domain contains a characteristic sequence motif. The 112amino acid %%%glycosyltransferase%%% 1 (GT1) motif represents the first half of the catalytic unit and contains a short aspartate-any residue-histidine (DXH) or aspartate-any residue-aspartate (DXD)-like sequence. Secondary structure predictions and structural threading suggest that the GT1 motif forms a 5-stranded parallel beta-sheet flanked by 4 alpha-helices, which resembles the first domain of the lactose repressor. Four invariant carboxylates and a histidine residue are predicted to lie at the C-terminal end of three beta-strands and line the active site cleft. Site-directed mutagenesis of murine ppGaNTase-T1 reveals that conservative %%%mutations%%% at these 5 positions result in products with no detectable enzyme activity (D156Q, D209N, and H211D) or <1% activity (E127Q and E213Q). The second half of the catalytic unit contains a DXXXXXWGGENXE motif (positions 310-322) which is also found in beta1,4-galactosyltransferases (termed the Gal/GalNAc-T motif). Mutants of carboxylates within this motif express either no detectable activity, 1% or 2% activity (E319Q, E322Q, and D310N, respectively). Mutagenesis of highly conserved (but not invariant) carboxylates produces only modest alterations in enzyme activity. %%%Mutations%%% in the C-terminal 128-amino acid %%%ricin%%%-like lectin motif do not alter the enzyme's catalytic properties.

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DIALOG(R)File 5:Biosis Previews(R)

14798281 BIOSIS NO.: 199900057941
Genetic defect in N-acetylglucosaminyltransferase I gene of a %%%ricin%%% -resistant baby hamster kidney mutant
AUTHOR: Opat Andrew S; Puthalakath Hamsa; Burke Jo; Gleeson Paul A (Reprint)
AUTHOR ADDRESS: Dep. Pathol. and Immunol., Monash Univ. Med. Sch., Commercial Road, Prahran, VIC 3181, Austria**Austria
JOURNAL: Biochemical Journal 336 (3): p593-598 Dec. 15, 1998 1998
MEDIUM: print
ISSN: 0264-6021
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The analysis of %%%mutations%%% associated with %%%glycosylation%%%-defective cell lines has the potential for identifying critical residues associated with the activities of enzymes involved in the biosynthesis of glycoconjugates. A %%%ricin%%%-resistant (RicR) baby hamster kidney (BHK) cell mutant, clone RicR14, has a deficiency in N-acetylglucosaminyltransferase I (GlcNAc-TI) activity and as a consequence is unable to synthesize complex and hybrid N-glycans. Here we show that RicR14 cells transfected with wild-type GlcNAc-TI regained the ability to synthesize complex N-glycans, demonstrating that the %%%glycosylation%%% defect of RicR14 cells is due solely to the lack of GlcNAc-TI activity. With the use of specific antibodies to GlcNAc-TI, RicR14 cells were shown to synthesize an inactive GlcNAc-TI protein that is correctly localized to the Golgi apparatus. We have cloned and sequenced the open reading frame of GlcNAc-TI from parental BHK and RicR14 cells. A comparison of several RicR14 cDNA clones with the parental BHK GlcNAc-TI sequence indicated the presence of two different RicR 14 cDNA species. One contained a premature stop codon at position +81, whereas the second contained a point %%%mutation%%% in the catalytic domain of GlcNAc-TI resulting in the amino acid substitution Gly320 fwdarw Asp. The introduction If a Gly320fwdarwAsp Asp %%%mutation%%% into wild-type rabbit GlcNAc-TI resulted in a complete loss of activity; the GlcNAc-TI mutant was correctly localized to the Golgi, indicating that the inactive GlcNAc-TI protein was transport-competent. Gly320 is conserved in GlcNAc-TI from all species so far examined. Overall these results demonstrate that Gly320 is a critical residue for GlcNAc-TI activity.

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DIALOG(R)File 5:Biosis Previews(R)

13222381 BIOSIS NO.: 199698690214
Characterization of single site %%%ricin%%% toxin B chain mutants
AUTHOR: Frankel Arthur (Reprint); Tagge Edward; Chandler John; Burbage Chris; Hancock Greg; Vesely Joseph; Willingham Mark
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JOURNAL: Bioconjugate Chemistry 7 (1): p30-37 1996 1996
ISSN: 1043-1802
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: DNA encoding %%%ricin%%% B chain was modified by site-directed mutagenesis, and eight separate mutant RTB cDNAs including four novel mutants were ligated into the baculovirus transfer vector, pAcGP67A. Cotransfection of S. frugiperda Sf9 cells with BaculoGold DNA was followed by limiting dilution isolation of recombinant baculoviruses. Infection of Sf9 cells at a multiplicity of infection of 5 in the presence of 25 mM lactose produced 0.05-1 mg/L of soluble, %%%glycosylated%%% 34 kDa proteins immunoreactive with monoclonal and polyclonal antibodies to %%%ricin%%% B chain. Mutant %%%ricin%%% B chains were partially purified by monoclonal antibody immunoaffinity chromatography to 10-50% purity in near milligram quantities. The mutant %%%ricin%%% B chains had decreased lectin binding relative to plant %%%ricin%%% B chain as measured by binding to immobilized lactose and asialofetuin and cell binding immunofluorescence. The mutant %%%ricin%%% B chains reassociated with plant RTA similarly to plant RTB, and the recombinant heterodimers had slightly reduced cell cytotoxicity relative to %%%ricin%%%.

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DIALOG(R)File 5:Biosis Previews(R)

13164344 BIOSIS NO.: 199698632177
Lec32 Is a new %%%mutation%%% in Chinese hamster ovary cells that essentially abrogates CMP-N-acetylneuraminic acid synthetase activity
AUTHOR: Potvin Barry; Raju T Shantha; Stanley Pamela (Reprint)
AUTHOR ADDRESS: Dep. Cell Biol., Albert Einstein Coll. Medicine, 1300 Morris Park AVe., New York, NY 10461, USA**USA
JOURNAL: Journal of Biological Chemistry 270 (51): p30415-30421 1995 1995
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: LEC29.Lec32 is a %%%glycosylation%%% mutant that was isolated from a selection of mutagenized Chinese hamster ovary (CHO) cells for lectin resistance. Compared with LEC29 CHO cells, the double mutant exhibited an unusually high sensitivity to the toxic lectin, %%%ricin%%%, indicating increased exposure of galactose residues on cell surface carbohydrates. Structural analysis of LEC29.Lec32 cellular glycoproteins showed a nearly complete lack of sialic acid residues. Genetic analysis demonstrated that the lec32 %%%mutation%%% is recessive and novel. Biochemical analysis showed that the mutant cells contained less than 5% of the cytidine 5'-monophosphate N-acetylneuraminic acid (CMP-NeuAc) present in parental CHO cells (1.6 nmol/mg of cell protein). A sensitive radiochemical assay used to measure CMP-NeuAc synthetase activity showed that the properties of this enzyme in parental CHO cells were essentially identical to those of CMP-NeuAc synthetase in various mammalian tissues. However, no CMP-NeuAc synthetase activity was detected in LEC29.Lec32 extracts. Mixing experiments provided no evidence for an inhibitor in the mutant CHO cells, and two revertants, which expressed only the LEC29 phenotype, had normal CMP-NeuAc synthetase levels. The combined evidence indicates that the lec32 %%%mutation%%% resides in either the structural gene encoding CMP-NeuAc synthetase or in a gene that regulates the production of active enzyme.

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DIALOG(R)File 5:Biosis Previews(R)

13025718 BIOSIS NO.: 199598493551
%%%Mutational%%% Analysis of the Ricinus Lectin B-chains: Galactose-binding ability of the 2-gamma subdomain of Ricinus communis agglutinin B-chain
AUTHOR: Sphyris Nathalie; Lord J Michael (Reprint); Wales Richard; Roberts Lynne M
AUTHOR ADDRESS: Dep. Biol. Sci., Univ. Warwick, Coventry CV4 7AL, UK**UK
JOURNAL: Journal of Biological Chemistry 270 (35): p20292-20297 1995 1995
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Ricin%%% B-chain (RTB) is a galactose-specific lectin that folds into two globular domains, each of which binds a single galactoside. The two binding sites are structurally similar and both contain a conserved tripeptide kink and an aromatic residue that comprises a sugar-binding platform. Whereas the critical RTB residues implicated in lectin activity are conserved in domain 1 of Ricinus communis agglutinin (RCA) B-chain, the sugar platform aromatic residue Tyr-248 present in domain 2 of RTB is replaced by His in RCA B-chain. In this study, key residues in the vicinity of the binding sites of the Ricinus lectin B-chains were altered by site-directed mutagenesis. The recombinant B-chains were produced in Xenopus oocytes in soluble, stable, and core-%%%glycosylated%%% forms. Both sites of RCA B-chain must be simultaneously modified in order to abolish lectin activity, indicating the presence of two independent, functional binding sites/molecule. Activity associated with the domain 2 site of RCA B-chain is abrogated by the conversion of Trp-258 to Ser. Moreover, the domain 2 site appears responsible for a weak binding interaction of recombinant RCA B-chain with GalNAc, not observed with native tetrameric RCA. Finally, the introduction of His at position 248 of RTB severely disrupts but does not abolish GalNAc binding.

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DIALOG(R)File 5:Biosis Previews(R)

11956712 BIOSIS NO.: 199396121128
Antisense and sense cDNA expression cloning using autonomously replicating vectors and toxic lectin selection
AUTHOR: Cummings Laura; Warren Charles E; Granovsky Maria; Dennis James W
AUTHOR ADDRESS: Samuel Lunenfeld Res. Inst., Mt. Sinai Hosp., 600 University Ave., Toronto, Ontario M5G 1X5, Canada**Canada
JOURNAL: Biochemical and Biophysical Research Communications 195 (2): p 814-822 1993
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: CHOP2 cells, a subline of the Chinese hamster ovary (CHO) cell %%%glycosylation%%% mutant Lec2 which expresses polyoma virus large T antigen,was used as the host cell line to select cDNAs conferring resistance to the toxic effects of %%%ricin%%%. Glycoconjugates in CHOP2 cells are deficient in sialic acid and therefore the cells are hypersensitive to %%%ricin%%%, a galactose-binding lectin. CHOP2 cells acquiring cDNA that either corrected the Lec2 %%%mutation%%% or created a Lec2/Lec1 phenotype were expected to show a selective growth advantage in %%%ricin%%%-containing medium. After a single cycle of transfection with a lymphoid cDNA library in pCDM8 followed by %%%ricin%%% selection, the predominant cDNA recovered from the cells was antisense N-acetyl-glucosaminyltransferase I (GlcNAc-TI) which conferred a Lec2/Lec1 phenotype. cDNA encoding GlcNAc-TI in a sense orientation was also enriched by transfecting a cDNA library into CHOP-1 cells, a mutant deficient in this enzyme, and selecting in medium containing ConA lectin. The results show that cell selection with toxic agents can be used to expression-clone both antisense and sense cDNA sequences from bi-directional cDNA libraries in the pCDM8.

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DIALOG(R)File 5:Biosis Previews(R)

11847530 BIOSIS NO.: 199396011946
%%%Ricin%%%-resistant Madin-Darby canine kidney cells missort a major endogenous apical sialoglycoprotein
AUTHOR: Le Bivic Andre (Reprint); Garcia Martine; Rodriguez-Boulan Enrique
AUTHOR ADDRESS: Biol. Differenciation Cellulaire, Unite de Recherche associee 179, Fac. des Sciences de Luminy, 13288 Marseille Cedex 09, France**France
JOURNAL: Journal of Biological Chemistry 268 (10): p6909-6916 1993
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: gp114 is a major sialoglycoprotein expressed on the apical membrane of Madin-Darby canine kidney (MDCK) II cells. We investigated its distribution in two lectin-resistant mutant cell lines derived from MDCKII cells, MDCKII-RCA-r and MDCKII-ConA-r cells. gp114 was present on the apical membrane of MDCKII-ConA-r cells but was predominantly basolateral in MDCKII-RCA-r cells. No change of polarity was observed for several apical and basolateral markers in this cell line. Reversal of polarity of gp114 mainly resulted from a modification of its intracellular sorting. gp114 showed altered endocytosis in MDCKII-RCA-r cells. In MDCKII cells gp114 was slowly endocytosed, whereas in MDCKII-RCA-r cells endocytosis of gp114 was highly increased. Using mannosidase I and II inhibitors we found that N-%%%glycosylation%%% only slightly affects gp114 sorting and endocytosis. Our results suggest that gp114 or an associated component in MDCK-RCA-r fails to express apical information or that a %%%mutation%%% creates a basolateral sorting signal which could be related to endocytic signals.

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DIALOG(R)File 5:Biosis Previews(R)

11171622 BIOSIS NO.: 199293014513
A SUBCLASS OF CELL SURFACE CARBOHYDRATES REVEALED BY A CHO MUTANT WITH TWO %%%GLYCOSYLATION%%% %%%MUTATIONS%%%
AUTHOR: STANLEY P (Reprint); SUNDARAM S; SALLUSTIO S
AUTHOR ADDRESS: DEP CELL BIOL, ALBERT EINSTEIN COLL MED, 1300 MORRIS PARK AVE, BRONX, NY 10461, USA**USA
JOURNAL: Glycobiology 1 (3): p307-314 1991
ISSN: 0959-6658
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A novel lectin-resistance phenotype was displayed by a LEC10 Chinese hamster ovary (CHO) cell mutant that was selected for resistance to the erythroagglutinin, E-PHA. Biochemical and genetic analyses revealed that the phenotype results from the expression of two %%%glycosylation%%% %%%mutations%%%, LEC10 and lec8. The LEC10 %%%mutation%%% causes the appearance of N-acetylglucosaminyltransferaseIII (GlcNAc-TIII) activity and the production of N-linked carbohydrates with a bisecting GlcNAc residue. The lec8 %%%mutation%%% inhibits translocation of UDP-Gal into the Golgi lumen and thereby dramatically reduces galactosylation of all glyuconjugates. This reduction in galactose addition does not, however, cause Lec8 mutants to be very resistant to the galactose-binding lectin, %%%ricin%%%. By contrast, the double LEC10.Lec8 behaved like a LEC10 mutant and was highly resistant to %%%ricin%%%. Based on structural studies of cellular glycopeptides as well as glycopeptides of the G glycoprotein of vesicular stomatitis virus grown in mutant cells, it appears that the %%%ricin%%% resistance of LEC10.Lec8 cells is due to the presence of a small number of Gal residues on branched, N-linked carbohydrates that also carry the bisecting GlcNAc residue. Labelling of N-linked cellular carbohydrates with [3H]galactose was found to occur at a low level for a wide spectrum of cellular glycoproteins in independent Lec8 mutants. Studies of the LEC10.Lec8 mutant have, therefore, led to the identification of a subset of structures that are acceptors for Gal when intra-Golgi UDP-Gal levels are limiting. This mutant also illustrates the potential for regulating cell surface recognition by carbohydrate-binding proteins by altering the expression of a single %%%glycosyltransferase%%% such as GlcNAc-III.

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DIALOG(R)File 5:Biosis Previews(R)

11168874 BIOSIS NO.: 199293011765
%%%MUTATIONAL%%% ANALYSIS OF THE GALACTOSE BINDING ABILITY OF RECOMBINANT %%%RICIN%%% B CHAIN
AUTHOR: WALES R (Reprint); RICHARDSON P T; ROBERTS L M; WOODLAND H R; LORD J M
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JOURNAL: Journal of Biological Chemistry 266 (29): p19172-19179 1991
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: %%%Ricin%%% B chain (RTB) is an N-%%%glycosylated%%% galactose-specific lectin which folds into two globular domains. Each domain binds one galactoside. The x-ray crystallographic structure has shown that the two binding sites are structurally similar and contain key binding residues which hydrogen bond to the sugar, and a conserved tripeptide, Asp-Val-Arg. We have used oligonucleotide site-directed mutagenesis to change either the binding residues or the homologous tripeptide in one or other or in both of the sites. The 5' signal sequence and RTB coding region were excised from preproricin cDNA and fused in frame to generate preRTB cDNA. Transcripts synthesized in vitro from wild-type or mutant preRTB cloned into the Xenopus transcription vector pSP64T using SP6 RNA polymerase, were microinjected into Xenopus oocytes. The recombinant products were segregated into the oocyte rough endoplasmic reticulum and core-%%%glycosylated%%%, and the N-terminal signal peptide was removed. %%%Mutating%%% sugar binding sites individually did not abrogate the lectin activity of RTB. When both sites were changed simultaneously, RTB was produced which was soluble and stable but no longer able to bind galactose. Changing the Asn residues of the two RTB N-%%%glycosylation%%% sites to Gln showed that oligosaccharide side chains were essential for both the stability and biological activity of recombinant RTB.

10277648 BIOSIS NO.: 199090062127
LECTIN-RESISTANT CHO CELLS SELECTION OF SEVEN NEW MUTANTS RESISTANT TO %%%RICIN%%%
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JOURNAL: Somatic Cell and Molecular Genetics 16 (3): p211-224 1990
ISSN: 0740-7750
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: In attempts to isolate new CHO %%%glycosylation%%% mutants, selection protocols using plant lectins that bind galactose residues of cell surface carbohydrates were applied to mutagenized CHO populations. The lectins were used alone or in combination to obtain seven %%%ricin%%% -resistant phenotypes. Each mutant had distinctive properties compared with previously described %%%ricin%%%-resistant CHO cells. One of the new phenotypes was dominant in somatic cell hybrids, and the others were recessive. Complementation analyses between related lectin-resistant (LecR) phenotypes indicated that each new isolate represented a novel genotype. Five of the mutants had properties typical of new CHO %%%glycosylation%%% mutants. The remaining two mutants were not readily categorized. Although they did not appear to be %%%ricin%%% -internalization or protein-synthesis mutants, they also did not display the marked alterations in sensitivity to several lectins of different sugar specificity expected for %%%glycosylation%%% mutants. The seven new LecR mutants described in these studies brings the total number of

different LecR CHO mutants isolated by this and other laboratories to about 40. Criteria for identifying new LecR %%%mutations%%% in CHO cells are discussed.

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DIALOG(R)File 5:Biosis Previews(R)

06903745 BIOSIS NO.: 198375087688
LECTIN RESISTANT MUTANTS OF POLARIZED EPITHELIAL CELLS
AUTHOR: MEISS H K (Reprint); GREEN R F; RODRIGUEZ-BOULAN E J
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JOURNAL: Molecular and Cellular Biology 2 (10): p1287-1294 1982
ISSN: 0270-7306
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Two lectin-resistant mutants derived from Madin Darby canine kidney cells, with constitutive alterations in the Asn-linked carbohydrate moieties, retained the characteristic structural and functional epithelial polarity of the parental cells. A %%%ricin%%% -resistant cell line was unable to incorporate galactose-sialic acid into glycoproteins and, from the pattern of cross-resistance to other lectins, appears to be different from previously described lines resistant to this lectin; the %%%mutation%%% in a concanavalin A-resistant line results, probably, in the production of defective carbohydrate cores of glycoproteins. In spite of %%%glycosylation%%% defects which result in an increased electrophoretic mobility of many cellular glycoproteins, both mutants retained the typical asymmetric structure of the plasma membrane (microvilli on the apical surface, junctional elements on the basolateral surface), functional tight junctions, and unidirectional active transport of electrolytes and water. Glycoproteins with terminal galactose-sialic acid moieties are apparently not critically involved in the development and maintenance of polarity in epithelial cells. The mutant cells, particularly the %%%ricin%%%-resistant line, exhibited morphological and electrophysiological changes which suggest a quantitative effect of the %%%mutations%%% on intracellular traffic of membranes and tight junction formation. The cell lines described the first lectin-resistant mutants of epithelial lineage, should prove useful tools for studying the peculiarities of %%%glycosylating%%% pathways in polarized cells.
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S2	17	S1 AND MUTAT?

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$47.09 17 Types
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$1.62 TELNET
$53.25 Estimated cost this search
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